



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Behlke et al.

Art Unit: 1634

Application No. 09/497,943

Examiner: Bradley L. Sisson

Filed: February 4, 2000

For: Primer extension methods for
production of high specific activity
nucleic acid probes

AMENDMENTS TO CLAIMS MADE IN RESPONSE TO
OFFICE ACTION DATED APRIL 23, 2002

Amendments to existing claims:

29. (Amended) A method of labeling a nucleic acid molecule, comprising the steps of:

- a. hybridizing a first nucleic acid to a second nucleic acid, wherein the first nucleic acid comprises, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - i. the Substrate Hybridization Domain [comprises] consists of a sequence of [about 5 to about 20] less than 10 nucleotides; and
 - ii. the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;

and the second nucleic acid comprises from 3' to 5': a Template Hybridization Domain and a Target Binding Domain, wherein:

- i. the Template Hybridization Domain [comprises] consists of a sequence of [about 5 to about 20] less than 10 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
- ii. the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain;

and:

- b. extending the second nucleic acid with a DNA polymerase in the presence of a labeled nucleotide to create an oligonucleotide having from 5' to 3' an unlabeled Target Binding Domain, a Template Hybridization Domain, and a labeled Signal Domain having a sequence which shows complementarity toward and is hybridizable to the Signal Template Domain[, thereby labeling said second nucleic acid molecule.]
30. The method of claim 29, wherein the nucleotides which comprise the first or second nucleic acid are deoxyribonucleotides.
31. The method of claim 29, wherein the nucleotides which comprise the first or second nucleic acid are ribonucleotides.
32. The method of claim 29, wherein the second nucleic acid consists of about 15 to about 150 nucleotides.
33. The method of claim 29, wherein the Substrate Hybridization Domain is at the 3' end of the first nucleic acid.
34. (Amended) The method of claim 29, wherein the Substrate Hybridization Domain [comprises] consists of a sequence of about 5 to [about] less than 10 nucleotides.
35. The method of claim 29, wherein the Substrate Hybridization Domain cannot be extended by a 5'→3' DNA polymerase.
36. The method of claim 35, wherein the Substrate Hybridization Domain further comprises an extension of nucleotides at the 3' end of said Substrate Hybridization Domain, the extension having no complementarity to the Template Hybridization Domain of the second nucleic acid.

37. The method of claim 35, wherein the Substrate Hybridization Domain comprises a 3'-terminal modified nucleotide.

38. The method of claim 37, wherein the modification is selected from the group consisting of: a 3'-amino-modifier, a 2', 3'-dideoxynucleotide, a 3'-phosphate, and a modified 3'-phosphate group.

39. The method of claim 37, wherein the Substrate Hybridization Domain comprises at least one nucleotide which comprises a modified cytidine, which nucleotide is selected from the group consisting of: C5-methyl-dC and C5-propynyl-dC.

40. The method of claim 29, wherein the Signal Template Domain comprises a sequence of about 10 to about 50 nucleotides.

41. The method of claim 29, wherein the Signal Domain is at least 50%, at least 70%, at least 90% or 100% homopolymeric.

42. [deleted]

43. The method of claim 29, wherein at least 60% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof, and the Signal Domain is at least 50% homopolymeric.

44. The method of claim 29, wherein the extending step is carried out by a DNA polymerase selected from the group consisting of: *E. coli* DNA polymerase I holoenzyme, Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase, T7 DNA polymerase, and a DNA polymerase encoded by a thermophilic bacterium.

45. The method of claim 29, wherein the Template Hybridization Domain or the Substrate Hybridization Domain comprises at least one modified nucleotide, which modified nucleotide increases the hybridization affinity of said Template Hybridization Domain to said Substrate Hybridization Domain.

46. The method of claim 45, wherein at least one modified nucleotide is found in the Template Hybridization Domain.

47. The method of claim 46, wherein at least one modified nucleotide is selected from the group consisting of: C5-methyl-dC, C5-propynyl-dC, C5-propynyl-dU, and 2,6-diaminopurine.

48. The method of claim 29, wherein at least one nucleotide comprises a label selected from the group consisting of: ^{32}P , ^{33}P , ^{35}S , fluorescein, digoxigenin, biotin, Cy5, Cy3, and rhodamine.

49. (Amended) A method for detecting a Target Nucleic Acid in a sample, comprising:

a. contacting the sample with a Complex under conditions whereby said Complex can bind to the Target Nucleic Acid to form a Complex-Target Nucleic Acid hybrid; wherein said Complex comprises:

i. a first nucleic acid comprising, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:

- (1) the Substrate Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides; and
- (2) the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;

and

ii. a second nucleic acid comprising from 3' to 5': a Signal Domain, a Template Hybridization Domain and a Target Binding Domain, wherein:

- (1) the Signal Domain comprises a sequence of about 5 to about 100 nucleotides, which sequence shows complementarity toward and is hybridizable to the

Signal Template Domain of the first nucleic acid, and of which at least two nucleotides are detectably labeled;

- (2) the Template Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
- (3) the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain;

and

- b. detecting any Complex-Target Nucleic Acid hybrids, so that if a Complex-Target Nucleic Acid hybrid is detected, a Target Nucleic Acid is detected in the sample.

50. (Amended) A method for detecting a Target Nucleic Acid in a sample, comprising:

- a. dissociating a Complex to generate a single stranded first nucleic acid and a single stranded second nucleic acid; wherein said complex comprises:
 - i. a first nucleic acid comprising, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - (1) the Substrate Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides; and
 - (2) the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;
 - and
 - ii. a second nucleic acid comprising from 3' to 5': a Signal Domain, a Template Hybridization Domain and a Target Binding Domain, wherein:

- (1) the Signal Domain comprises a sequence of about 5 to about 100 nucleotides, which sequence shows complementarity toward and is hybridizable to the Signal Template Domain of the first nucleic acid, and of which at least two nucleotides are detectably labeled;
 - (2) the Template Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
 - (3) the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain;
- b. contacting the sample with the second nucleic acid of step a. under conditions whereby said second nucleic acid can bind to the Target Nucleic Acid to form a second nucleic acid-Target Nucleic Acid hybrid; and
 - c. detecting any second nucleic acid-Target Nucleic Acid hybrids, so that if a second nucleic acid-Target Nucleic Acid hybrid is detected, a Target Nucleic Acid is detected in the sample.

51. (Twice amended) A kit for labeling a nucleic acid molecule, comprising a reaction mixture and a DNA polymerase, wherein the reaction mixture comprises:

- a. a first nucleic acid comprising, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - i. the Substrate Hybridization Domain [comprises] consists of a sequence of [about 5 to about 20] less than 10 nucleotides; and
 - ii. the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;and
- b. a second nucleic acid comprising from 3' to 5': a Template Hybridization Domain and a Target Binding Domain, wherein:

- i. the Template Hybridization Domain [comprises] consists of a sequence of [about 5 to about 20] less than 10 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
- ii. the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain[.];
- c. wherein the hybridization domains of the first and second nucleic acids hybridize to each other under conditions in which an enzyme can extend the second nucleic acid by adding a sequence complementary to the Signal Template Domain.

52. The kit of claim 51, wherein at least 60% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof, and the Signal Domain is at least 50% homopolymeric.

53. The kit of claim 51, wherein the Substrate Hybridization Domain comprises a predetermined sequence comprising CCCGCC and the Signal Template Domain comprises a predetermined sequence comprising TTTTTTTTTT.

54. The kit of claim 51, wherein, the first nucleic acid comprises a predetermined sequence comprising SEQ ID NO:10.

55. The method of claim 29, wherein the Probe has a specific activity of at least 7×10^7 CPM per picomole, and wherein the Probe comprises the Target Binding Domain, the Template Hybridization Domain and the Signal Domain.

56. The method of claim 29, wherein the Probe has a specific activity of at least 9×10^7 CPM per picomole, and wherein the Probe comprises the Target Binding Domain, the Template Hybridization Domain and the Signal Domain.

57. (new) The method of claim 29, wherein the first nucleic acid has a hairpin loop disposed to the 5'-side of the Signal Template Domain.